The Examiner under paragraph 4 and 5 rejected claim 25 of the instant application. Because this is a provisional rejection, the Applicant will address it when one of the allegedly conflicting claims has been patented.

The Examiner under paragraph 6 and 7 rejected claim 25 of the instant application, based on 35 USC § 112. Applicant points out that the Examiner provided no reasoning for the conclusion that the Applicant specification failed to reasonably convey the claimed invention. An application is presumed to satisfy section 112 unless reasoning is presented that shows that is not the case. Here, the use of the word "comprising" simply cannot be the reason, as discussed below. Therefore, the Applicant respectfully requests that the rejection be withdrawn.

The term "comprising" in conjunction with claims for nucleic acid probes for *Escherichia coli* and Shigella has been used routinely in previously issued patents. See, for example, Lampel et al., "Probe to Identify Enteroinvasive *E. coli* and Shigella Species (5,041,372) claim 2, and Parodos et al., "Probes for the Specific Detection of *Escherichia coli* and Shigella (5,084,565) claim 4.

Further, the need for disclosing a representative number of the species of the isolated nucleic acid sequences encompassed in claim 25 is not apparent from patents for DNA probes for *Escherichia coli* and Shigella that have issued, specifically Lampel and Parodos cited above. Applicant therefore respectfully requests the withdrawal of the rejection.

The Examiner under paragraphs 8, 9, and 10 rejected claim 25 of the instant application based on 35 USC § 102. However, claim 25 recites nucleic acid probes capable of distinguishing certain species.

The Examiner under paragraphs 8, 9, and 10 rejected claim 25 of the instant application based on 35 USC § 102. However, claim 25 recites nucleic acid probes capable of

distinguishing certain species. Nowhere does the reasoning for these rejections explain how the cited documents meet this element of distinguishing species or between species. Even if the cited documents discuss sequences that could comprise a sequence as recited in claim 25, they nowhere discuss how to produce sequences capable of distinguishing species. In fact, as explained previously and again below, Cilia actually notes a failure to distinguish species with the sequences discussed in his paper. An anticipatory document must disclose all the elements of a claim. Obviously, this element has either not been discussed in the cited documents or discussed in only the failure to meet this element. This rejection is in error and applicant respectfully requests its withdrawal.

The Applicant will respond to the provisional rejection of paragraph 11 when one of the co-pending applications is allowed.

The Examiner at paragraph 12 has rejected claim 25 under 35 U.S.C 102(f). Frank Portugal is the inventor of the claimed subject material for 09/027,089. The Examiner has not suggested any reason why this is inconsistent with the inventorship in another application.

In response to the rejection under 35 USC § 103, paragraph no 13, Applicant submits that the following arguments. Applicant respectfully requests withdrawal of the rejection.

Comments on the Examiner Response to Arguments

The invention of Portugal should be patentable over the combination of prior art of Hammond, Hogan, Anderson, Dyson and Cilia and should not be rejected under 35 U.S.C. 103(a) because taken together these citations fail to teach the use of a combination of wash temperatures above the T_m. Arguments against Hammond, Hogan, and Anderson were previously overcome by the Applicant as described in the January 22, 2001, Reply and Amendment. Collectively, the citations by the Examiner fail to teach the use of wash temperatures that exceed the T_m.

The Examiner's position in summary is that methods of using probes to identify closely related organisms were well known in the art at the time of the invention as well as manipulations of reaction conditions to increase stringency. Thus, one skilled in the art would from the prior art know elevation of washing above the Tm. The Applicant's position is that washing above the T_m is not just an extension of the prior art and would not be known by one skilled in the art. The Applicant respectfully submits four arguments, among those possible, in support of his position:

1. The prior art specifically explains and teaches why the T_m should *not* be approached or exceeded when washing hybridized strands, in contradiction to the instant invention.

2. The patent and scientific literature show that these teachings have been strictly adhered to and that there is no evidence that anyone has previously exceeded the calculated T_m for a wash.

3. The patent and scientific literature show that numerous efforts have been made to separately identify *E. coli* and the four species of Shigella without success despite access to and a full knowledge of the prior art cited by the Examiner.

4. The scientific literature claims priority for the separate identification of *E. coli* and Shigella, which occurred after the instant invention.

The Examiner maintains that altering wash temperatures so that they extend above the T_m is an obvious extension of the prior art by one skilled in the art. However, the invention of Portugal is not obvious over the prior art. If wash temperatures above the T_m were obvious over the prior art, then evidence of such washes would be found in prior patents or publications. In the past 40 years of development and usage of hybridization methodology, based on the scientific literature, there does not appear to be any evidence for hybridization above the T_m, with the exception of the publication by Sabat et al.

previously cited in the January 22, 2001, Reply and Amendment in support of the invention of Portugal, and which occurred after the instant invention.

In support of the above statement, the following evidence is provided. Fifteen major manuals on hybridization methods and papers were surveyed in the library at the National Institutes of Health. These manuals cover a 16-year period from 1985-2001 in a diverse set of fields, including the following:

- In situ (J.E. Beesley, *Immunocytochemistry and In Situ Hybridization in the Biomedical Sciences,* Birkhauser, Boston, 2001)
- Diagnostic molecular microbiology (D.H. Persing, T.F. Smith T.F., F.C. Tenover, and T.J. White, "*Diagnostic Molecular Microbiology,*" American Society for Microbiology, Washington, D.C., 1993)
- In situ (T. Schwarzacher and P. Heslop-Harrison, "*Practical* in situ *Hybridization,* Springer, Heidelberg, Germany, 1999)
- Bacterial systematics (E. Stackebrandt and M. Goodfellow, "*Nucleic Acid Techniques in Bacterial Systematics,*" John Wiley & Sons, Chichester, England, 1991)
- In situ (J.M. Polak and J. O'D McGee, "In Situ *Hybridization: Principles and Practice, Second Edition,*" Oxford University Press, England, 1998)
- Recombinant DNA technology (J.J. Greene and B.V.B. Rao, "*Recombinant DNA Principles and Methodologies,*" Marcel Dekker, New York, 1998)
- In situ (N. Harris and D.G. Wilkinson, "In Situ *Hybridisation: Application to Developmental Biology and Medicine,*" Cambridge University Press, Cambridge, England, 1990)
- Nucleic acids (R. Rapley, *The Nucleic Acid Protocols Handbook,*" Humana Press, Totowa, New Jersey, 2000)
- In situ (G.R. Coulton and J de Belleroche, "In Situ *Hybridization: Medical Applications,*" Kluwer Academic Publishers, Boston, Massachusetts, 1992)
- DNA probes (G.H. Keller and M.M. Manak, "*DNA Probes,*" Stockton Press, New York, 1993)

- In Situ (D.G. Wilkinson, "In Situ *Hybridization: A Practical Approach,*" Oxford University Press, Oxford, England, 1992)
- Nucleic acid hybridization (B.D. Hames and S.J. Higgins, "*Nucleic Acid Hybridisation: A Practical Approach,*" IRL Press, Oxford, England, 1985)
- In situ (M-F. Chesselet, "In Situ *Hybridization Histochemistry,*" CRC Press, Boca Raton, Florida, 1990)
- Gene probes (B.D. Hames and S.J. Higgins, "*Gene Probes 2: A Practical Approach,*" IRL Press, Oxford, England, 1995)

These manuals jointly list more than 5,000 references (see attachments). These manuals *do not disclose a single example of washing above the* T_m. To one skilled in the art, therefore, the paper of Sabat et. al. that reports hybridization *above the* T_m, and is useful for specifically differentiating between Shigella species, would therefore be recognized as exceptional, as the Applicant noted previously in the January 22, 2001, Reply and Amendment in support of the instant invention.

On page 11, the Examiner states (paragraph 1, line 3):

> "Dyson specifically teaches that oligonucleotides are hybridized at a temperature between 5 and 10 degrees below the T_m for 14-48 hours and that filters are then washed four times *at* the hybridization temperature. Dyson teaches that often, such a wash is enough; however, Dyson teaches that if the filters still show considerable activity above the background, the wash temperature should be increased by 2-3° C and the wash should be repeated."

The Examiner may be equating the hybridization temperature with the T_m. However, the two are quite different. The hybridization temperature is the temperature at which two strands of nucleic acid of known size and composition under carefully defined conditions will reassociate. In contrast, the T_m is defined as the point where 50 percent of the hybrid has *dissociated* (melted).

Since the initial development of hybridization in 1961, it has been rigorously asserted and accepted in the prior art and by those skilled in the art that the ideal temperature for hybridization is generally 20-25° C below the T_m, where theory predicts the maximal rate of hybridization occurs. Because the rate of hybridization according to the prior art actually *decreases* as one raises the hybridization temperature from 20-25° C below the T_m, one skilled in the art would based on the prior art never exceed the T_m. That is why the invention of Portugal meets the test of unobviousness.

The invention of Portugal is also unobvious because it reveals that when multiple and variant copies of ribosomal RNA genes are simultaneously probed, unanticipated and unexpected results occur. The invention of Portugal teaches that one must hybridize at a minimum of two different temperatures at or above the T_m to uncover the effects that can occur under this set of conditions. The invention of Portugal further teaches that when this is carefully done, advantage can be taken of the differential hybridization responses to enable one to create multiple flow diagrams. Use of such diagrams teaches one skilled in the art how to make very fine discriminations between species whose genomes may show 90 percent or greater homology.

The claims of the present invention address two or more washes at temperatures relative to the calculated or experimentally determined T_m of an oligonucleotide probe, not the hybridization temperature.

Furthermore, the Examiner submits that Table 2 of Dyson further teaches that it would have been readily apparent to one of ordinary skill in the art to increase wash temperatures by 2-3 degrees at a time, and to repeat until suitable hybridization had occurred (page 12, line 8). However, the Examiner has not fully disclosed the complete statement attributable to Dyson. Dyson further states as part of the same paragraph quoted above by the Examiner:

"The washing temperature can be gradually increased until the T_m is reached. The final wash should be at the T_m for 2 min only."

Thus, in contrast to the present invention, Dyson does not teach one skilled in the art to wash above the T_m. In contrast, the invention of Portugal addresses the need to vigorously wash under conditions that have not previously been recognized or used, including washing above the T_m and prolonged (10 min) washes at the T_m.

Furthermore, Table 2 of Dyson still does *not* teach the present invention. While hybridization and wash temperatures are presented, the compositions of the oligonucleotides in the hybridization reactions are not specified. As Dyson points out in Section 3.11, the T_m of the oligonucleotide will depend on the sequence composition, with 2° for each A or T nucleotide in the composition and 4° for each G or C. Thus, one skilled in the art cannot know from Table 2 the exact sequence composition of the oligonucleotides used and therefore cannot relate this information to the T_m being used. Therefore Table 2 cannot support the assertion, particularly with the quote from Dyson above on increasing the temperature **only** to the T_m and even then just briefly, that one skilled in the art would have reason or know to go beyond the specified T_m.

13. (a) Hammond

The Examiner states that Hammond teaches hybridization probe assays for *Chlamydia pneumonia* that can distinguish *C. pneumoniae* from its most closely related taxonomic or phylogenetic neighbors. As the Applicant noted in the Reply and Amendment of January 22, 2001, Hammond *fails* to teach the fine discrimination between closely related genus and species that is taught in the present invention. *C. pneumoniae* and *C. psittaci* show far greater sequence differences than do the *Shigella* species, so using DNA probes to differentiate between them is easier. Thus, differentiating between these two Chlamydia species is not evidence that the invention of Hammond can also differentiate between the much more closely related species of Shigella and *Escherichia coli.*

Nor does Hammond teach the *washing* of a hybridized complex at or above the T_m. While Hammond may teach the need for high stringency, nowhere does Hammond teach that high stringency would extend at or above the T_m. If washing above the T_m were obvious over the prior art, then examples would be seen in the literature. With the exception of Sabat et al., published after the date of the instant application, such references appear to be lacking. These points were raised previously in the Reply and Amendment of January 22, 2001.

The objective challenge of hybridization studies is to make discriminations as sensitive and specific as possible. That objective is extremely difficult to achieve because there may be only a single nucleotide difference between two organisms being probed with a 20-mer oligonucleotide. By contrast, making hybridizations as broadly inclusive as possible is much simpler. This merely entails finding identical regions for both organisms, of which for 16S ribosomal RNA of bacteria such as *Shigella* there are far more. Thus, the point of patents such as Hammond is to try to distinguish much closer-related species than *C. pneumoniae* and *C. psittaci*. If all it took to do this was to simply use the teachings found in the prior art then Hammond and the others cited in the Examiner's response would do it. Given the incentive to create a novel invention where others have not, *the fact that Hammond and others do not distinguish between very closely related organisms means that they cannot because the prior art fails to teach them how to do so.*

13. (b) Hogan

Similarly, the Applicant in Reply and Amendment of January 22, 2001, addressed the deficiencies of Hogan in teaching over the invention of Portugal. Like Hammond, Hogan fails to teach anywhere in the patent to wash at temperatures *at or above the* T_m. Consequently, Hogan fails to teach how to differentiate among closely related species. Most importantly, Hogan clearly demonstrates that the prior art does not teach the invention of Portugal.

Escherichia coli and the four *Shigella* species cause different infectious diseases, with different sequelae and treatment requirements. It is critical therefore to be able to differentiate between an *E. coli* infection and one caused by *Shigella.* Had the prior art taught how to make the fine discriminations needed, as the Examiner suggests it does by simple extension of the prior art, then Hogan would have done so. That Hogan's invention can only detect *E. coli* and *Shigella* together does little for the objectives of using hybridization in the first place to discriminate between species for clinical purposes. Hogan's invention specifically demonstrates that the prior art fails to teach the invention of Portugal.

13. (c) Anderson

The Examiner may have confused the stringency of hybridization temperature with the stringency of wash temperatures. Anderson does not teach or suggest *any wash temperatures* for individual oligonucleotides as was pointed out in the Applicant's January 22, 2001, response. Anderson does not recommend washing either at or above the T_m even for a mixture of oligonucleotide probes.

13 (d) Cilia

Cilia actually notes a failure to distinguish species with the sequences discussed in his paper. An anticipatory document must disclose all the elements of a claim. Obviously, this element has either not been discussed in the cited documents or discussed in only the failure to meet this element. This rejection is in error and the Applicant respectfully requests its withdrawal.

13 (e) Sabat

The requirements to publish a scientific paper may indeed differ from the criteria applied to the overcoming of 35 USC 103. However, one rarely finds statements of research findings that are claimed as "a first" as was found in the paper of Sabat et al. The reason

for this is that unless extremely clear cut, priority in the scientific literature is usually very hard to prove. Thus, the acceptance of the priority claim by a peer-reviewed journal whose reviewers very closely scrutinize such statements indicates that those skilled in the art themselves recognized that hybridization results that depended on temperatures above the T_m were previously unknown.

If the Examiner cites the scientific literature (Dyson, Hogan, Hammond, and Anderson, for example) to disallow the invention of Portugal over the prior art, then so should the Examiner admit the novelty of new art made by skilled practitioners and recognized as novel by those skilled in the art who review submissions prior to publication in scientific journals. The Examiner has rejected claims 19-24 under 35 USC 103. Therefore, the Applicant respectfully requests that this rejection be withdrawn.

The Examiner maintains that prior art methods at the time of the invention were sufficient to distinguish between very closely related bacteria as exemplified by *E. coli* and *Shigella*. The Examiner further cites Hogan as not achieving this not because of insufficient prior art but because of the use of different sequences. However, as the Applicant noted in the Reply and Amendment for the invention of Portugal on January 22, 2001, prior art was *not* available to Hogan because Cilia, which comes later, still failed to demonstrate that he could differentiate between *Shigella* and *E. coli* even though he specifically tried.

When Cilia attempted to differentiate between *Shigella* and *E. coli* he met the necessary criteria cited by the Examiner including (1) availability of the same set of sequences as found in the invention of Portugal and (2) all of the requisite details from the prior art of hybridization. Cilia relied on polymerase chain reaction (PCR) methods, which are presumed to be even more sensitive than the methods used in the invention of Portugal but which also incorporate a hybridization step. Had the prior art that existed before the instant invention was made been sufficient as claimed by the Examiner, then Cilia would have achieved the invention as well. The fact that Cilia fails supports the claim by the

invention of Portugal that the prior art was insufficient. Sabat et. al. succeeded whereas Cilia failed because Sabat et. al. incorporate the invention of Portugal.

Conclusion

The prior art does *not* teach one skilled in the art to exceed the T_m of the hybridized probe when washing. Rather, the prior art teaches just the opposite, that the ideal wash temperatures for any probe should be several degrees below the T_m (for example, Hogan, Hammond, and Anderson combined). Even Dyson fails to teach that the wash temperature has to exceed the T_m. Without the necessary teaching to wash for prolonged time at or above the T_m, one skilled in the art cannot make the instant invention.

The Examiner further states that the instant invention is not independent of sequence and process for distinguishing, because the specification in the instant application for *S. boydii*, for example, identifies all the organisms and not just *S. boydii*. The Examiner bases her expectations on the prior art. The Applicant has previously noted, however, that (1) the hybridizations of the instant invention are not targeting a single gene but a set of variants and (2) the results of the instant invention are not based on the prior art. Since the instant invention teaches new art, one should not expect the same results.

Thus, the instant application teaches that each *Shigella* organism, for example, has multiple operons for ribosomal RNA (rRNA) and that the sequences of these operons may deviate slightly from one another. The mix of sequences and operon combinations may also vary from one species of *Shigella* to another. When a rRNA gene is sequenced therefore, the resulting assemblage of nucleotides is a composite sequence. This accounts for the lack of consistency between the "presumed" rRNA sequence for each organism and how each derived probe behaves. If each *Shigella* organism had but a single gene for its rRNA, the independence of sequence would be readily apparent.

In addition, the Examiner suggests that only the exact steps in the flow diagram of the instant application would be allowable. However, the invention of Portugal does not

consist of just a single flow diagram for distinguishing between species of *Shigella* and between *Shigella* and *E. coli*. Rather, the instant application presents a general method for distinguishing between closely related bacterial species using at least two wash temperatures at or above the T_m. For example, using the subject matter of the instant invention, one of several alternate flow diagrams could comprise the following steps:

1. SEQ ID No. 3 at 70° C (Reaction: *E. coli*; no reaction *Shigella*)
2. SEQ ID No. 1 at 72° C (Reaction *S. sonnei*)
3. SEQ ID No. 2 at 62° C (Reaction *S. dysenteriae*)
4. SEQ ID No. 3 at 66° C (Reaction *S. flexneri)*
5. No reaction indicates *S. boydii*

The Applicant thanks the Examiner for the suggested allowance of revised claims and for the diligent responses to the various arguments.

Respectfully submitted,

Frank Portugal

October 22, 2001

Encl: payment form for three-month extension ($460.00)
Additional payment form

FAX

To: Commissioner for Patents

From: Frank Portugal, Ph.D.

Date: October 22, 2001

Pages (including cover): 15

Message: Certificate of Facsimile Transmission to Patent and Trademark Office

I certify that this document and its attachments (14 pages) is being transmitted to the Commissioner of Patents at fax number [(703) 305-3014], this 16th day of January, 2001.

Frank Portugal, Ph.D.
Application # 09/027,089